FDS8 (Oct 2019)

RGC Ref. No.: UGC/FDS17/M08/19 (please insert ref. above)

# RESEARCH GRANTS COUNCIL COMPETITIVE RESEARCH FUNDING SCHEMES FOR THE LOCAL SELF-FINANCING DEGREE SECTOR

# FACULTY DEVELOPMENT SCHEME (FDS)

## **Completion Report**

(for completed projects only)

Submission Deadlines:	1.	Auditor's report with unspent balance, if any: within six months of
		the approved project completion date.
	2.	Completion report: within <u>12</u> months of the approved project
		completion date.

# **Part A:** The Project and Investigator(s)

# 1. Project Title

Characterising and Fingerprinting Biomarkers of Urolithiasis: A Case Control Study (Stage 2 of 2)

# 2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	GOHEL, Mayur Danny I / Professor	MHS / Tung Wah College
Co-Investigator(s)	NG, Chi Fai / Professor	Surgery / CUHK
Others		

# 3. Project Duration

	Original	Revised	Date of RGC / Institution Approval (must be quoted)
Project Start Date	01/01/2020		09/09/2019
Project Completion Date	31/12/2022	30/06/2023	11/07/2022
Duration (in month)	36-months	42-months	11/07/2022
Deadline for Submission of Completion Report	31/12/2023	30/06/2024	11/07/2022

4.4 Please attach photo(s) of acknowledgement of RGC-funded facilities / equipment.

# Part B: The Final Report

#### 5. Project Objectives

- 5.1 Objectives as per original application
  - 1. To monitor the temporal changes of selected biomarkers (from stage 1) in recurrent stone-formers and relate biomarker changes to supporting clinical signs and data.
  - 2. To correlate the changes in cytokine expression levels with urolithiasis and/or other biological conditions or diseases.
  - 3. To gather the dietary and fluid-intake profiles of patients with recurrent stones during the course of the longitudinal study.
  - 4. To recommend a biochemical work-up protocol for use as a diagnostic tool to identify patients with a 'silent' stone forming.
  - 5. To train undergraduate or postgraduate students in biochemical, molecular and immunological techniques and to enhance the institution's research capacity.

#### 5.2 Revised objectives

Date of approval from the RGC:	6 November 2019
	Objective 3 was removed as funding for the
	Nutrition software was not approved and budget
Reasons for the change:	was cut by 45% including staffing, one of whom
	would have been dedicated to do the extensive
	survey and nutrition analyses.
1 77	

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- 3. To recommend a biochemical work-up protocol for use as a diagnostic tool to identify patients with a 'silent' stone forming.
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#### 5.3 Realisation of the objectives

(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

The project started at the beginning of 2020, during the early stages of CoVID epidemic and it was challenging both from manpower and recruitment of patients' aspects. The project recruited a research assistant (RA) in mid-2020, who left after 1-year and after several rounds of advertisements, it was difficult to recruit a suitable RA. Once the clinics opened in April 2022, the PI resorted to doing the collections and certain experiments, himself together with recruitment of  $(3^{rd} \text{ and } 4^{th} \text{ year})$  students from his programme to assist in processing of samples and conducting biochemical and ELISA assays for IL-6, IL-8, NGAL and Hyaluronan (HA). 6 undergraduate students were trained during this period. ( $\checkmark$  Objective 5).

As the clinic resumed in April 2022 and 4 quarters of sample collections remained, an extension was requested till 30 June 2023 (approved by the College on 11 July 2022). It was not until all samples were collected until December 2023. (Due to some of the preceding patients resuming in the study after clinic suspension during CoVID).

*Objective 1* was completed  $\checkmark$ , in January 2024 with all the biomarkers being done from the patients that were recruited since July 2020 to December 2023 (including suspension of clinic during December 2020 to April 2022). An inflammatory molecule, Hylauronan (HA), detectable in urine, was found to be useful biomarker in active and post-treated stone-formers in preliminary results. The overall temporal changes in patients following removal of the initial stone indicated that IL-8, HA and NGAL had an upward trend, suggesting a silent stone formation.

It was fortunate that the time was extended (from 24-months to 30 months), as during the study, some patients did develop recurrent stones and that gave insight to correlation of the biomarkers with the forming of silent stones and picking up sub-clinical conditions. (*Objective 2*  $\checkmark$ ). 9 patients had a recurrent episode of stone during the 30-months. In certain patients, the levels of oxalate were higher concomitantly with lower citrate levels (a protective ion that chelates calcium, thus preventing from further causing precipitation in the urinary tract.

This has facilitated to complete *Objective*  $4 \checkmark$ , which is the main overall objective of the study – that to identify a reliable biomarker in routine laboratory test to indicate a potential silent stone-forming. This objective is 80-90% complete as the PI is working on several complex algorithms (biomarkers, Imaging (ultrasound, spiral-CT), urine ion concentrations, patient profile (sex, age, first time / recurrent stone former), which will be incorporated in the publication being submitted shortly. (All data were finalized and completely analysed in January 2024).

The budget went underspent, mainly for two reasons (1) manpower and difficulty to recruit research personnel during the CoVID epidemic and (2) there were not many meetings / symposiums during 2020 – 2022 until one went ahead in the summer of 2022. This accounted for 39% of the budget being unspent (mainly manpower and conference). Nevertheless, the PI managed to complete the project with a pool of talented final year undergraduate students and PI conducting the principal laboratory work and analysis.

5.4 Summary of objectives addressed to date

<b>Objectives</b> (as per 5.1/5.2 above)	Addressed (please tick)	<b>Percentage Achieved</b> (please estimate)
1. To monitor the temporal changes of selected biomarkers (from stage 1) in recurrent stone-formers and relate biomarker changes to supporting clinical signs and data.	$\checkmark$	100%
2. To correlate the changes in cytokine expression levels with urolithiasis and/or other biological conditions or diseases.	$\checkmark$	100%
3. To recommend a biochemical work-up protocol for use as a diagnostic tool to identify patients with a 'silent' stone forming.	$\checkmark$	80-90%
4. To train undergraduate or postgraduate students in biochemical, molecular and immunological techniques and to enhance the institution's research capacity.	$\checkmark$	100%

#### 6. Research Outcome

# 6.1 Major findings and research outcome *(Maximum 1 page; please make reference to Part C where necessary)*

80 stone-formers and 50 normal controls were recruited for the 30-month (from 24-month) longitudinal study to measure the temporal changes (9 data collection points over 30 months) in lithogenic ions – oxalate, calcium, citrate and phosphate, biomarkers Hyaluronan (HA), urinary NGAL, IL-6 and IL-8. (The 4 biomarkers were short-listed from Stage-1 of the study which was completed December 2019).

The first principal biomarker that we extracted and were successful in measuring in the samples was HA which was found to be in higher content in active stone-formers and those developing new stones. The lithogenic ions oxalate and citrate were found to be high in stone-formers which is expected as the patients would be in citrate therapy. However, recovered stone-formers would see a drop in citrate and put them at risk of forming further recurrent stones. (*Preliminary results at 5th Experts in Stone Disease Conference; Full Completed data at Association of Diagnostics & Laboratory Medicine 2024*)

During the initial recruitment of the patients, it was notice by the research staff that patients would take Chinese medicine concomitantly with western medicine (if prescribed) following stone removal. To further investigate this, a side study beyond the objectives was done to survey the nature, type, and extent of such Chinese medicine. The preliminary results of this were presented at the plenary as a wider debate on the pros and cons of phytotherapy (*Plenary debate at 5th Experts in Stone Disease Conference*). This part of the project has a good potential for follow-up further studies.

After the completion of all laboratory results and analysis in January 2024, a clear trend in the biomarkers appeared. IL-8, HA and NGAL saw increasing trends in those stone-formers who were treated and entered the 30-month study. This is significant as there is possibility that early check and diagnosis can be made by simple urine test. (*Moderated oral poster at 44th Congress of the Societe International d'Urologie*)

On completion of all data collected in January 2024, a manuscript was submitted in March 2024 to Urolithiasis, which was returned for additional information and revision. A revised manuscript will be submitted in May 2024. A second manuscript is being written up for the data acquired on the use of traditional Chinese medicine (TCM) which gave some interesting results. This will be submitted to a comparable journal with the scope or TCM and treatment and / or prevention of diseases / illnesses, eg. *J Ethnopharmacology, J Altern Complement Med, Chinese Medicine*.

# 6.2 Potential for further development of the research and the proposed course of action (Maximum half a page)

This study conclusively shows potential diagnostic biomarkers, HA, NGAL & IL-8 that could be used in routine urinary tests for renal stone patients. This project only discovered that these 3 of the several others (9 potential biomarkers) were promising. This is further worth investigating in clinical trials to monitor those patients who have recovered and conduct these specific tests to discover if there is a silent stone forming. This would be more cost-effective than doing CT-scans as imaging costs would be much higher. The latter can be used when a thorough metabolic work-up and evaluation points to a silent stone.

One of the interesting aspects was the extent of Chinese medicine used by the patients. We found in our studies, almost 90% consulted a Chinese medicine practitioner and of those, 70% did take preventive Chinese medicines to treat renal stones. This was not part of the objective of the study, but it was a discovery when patients were asked about their fluid, salt, and general dietary habits. This study points to a new direction to investigate, especially when 90% of the patients do consult TCM practitioners.

Finally, the PI is working on algorithms on various aspects and variables to propose a metabolic work-up including the biomarkers that were investigated to propose to Urologists in their follow-up of idiopathic renal stone -formers. This would be completed by October 2024 and be presented at the international meeting (44th Congress of the Societe International d'Urologie) in New Delhi, India, where an abstract has been submitted.

#### 7. Layman's Summary

(Describe <u>in layman's language</u> the nature, significance and value of the research project, in no more than 200 words)

The main goal of this project was to investigate a simple, cost-effective test to monitor renal stone formers who become afflicted with unbearable pain (renal colic). It is a matter of time before another stone will develop in their lifetime. This project and its deliverables were to investigate if there is any viable biomarker that can be used for detecting a silent stone forming before renal colic appears. This project conclusively determines 3 urinary biomarkers (of 9 tested) that can potentially be used. They are two cytokines Interlukin-8, Hyaluronan and a renal damage marker NGAL. One other outcome of this project was, patients seeking TCM doctors for advice (90%). This needs further studies to the extent of advice, medicines taken, efficacies and any reactions with current medications. The outcome of this study may allow follow-up treatment to be instigated at the most appropriate times, thereby bringing both financial and health benefits. Annual health checks that include assessments (blood / urine) for various organ systems and general health is not unusual nowadays and this study envisages the addition of one more test to be included so that it (together with the renal panel) can provide useful information to the clinicians.

# Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising <u>Directly</u> From This Research Project (Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				Title and Journal / Book					
Year of Publication	Year of Acceptance (For paper accepted but not yet published)	Under Review	Under Preparation (optional)	Author(s) (denote the correspond- ing author with an asterisk*)	(with the volume, pages and other necessary publishing details specified)	Submitted to RGC (indicate the year ending of the relevant progress report)	Attached to this Report (Yes or No)	Acknowledged the Support of RGC (Yes or No)	Accessible from the Institutional Repository (Yes or No)
2024		√	¥	Gohel MDI* & Ng CF	Preliminary detection of biomarkers in recurrent renal stone formers / <i>Urolithiasis</i>		No	Yes	Yes
2024/2025			~	Gohel MDI*, Wong SC & Ng CF	The use of Traditional Chinese Medicine in renal stone formers / <i>TBD</i>		No	Yes	Yes

# 9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered

(Please attach a copy of each conference abstract)

Month / Year / Place	Title	Conference Name	Submitted to RGC (indicate the year ending of the relevant progress report)	Attached to this Report (Yes or No)	Acknowledged the Support of RGC (Yes or No)	Accessible from the Institutional Repository (Yes or No)
June 10-11, 2022 Athens, Greece.	Seeking reliable urinary biomarkers to indicate a forming stone	5 <sup>th</sup> Experts in Stone Disease Conference	2024	Yes	Yes	Yes
June 10-11, 2022 Athens, Greece	Debate – Phytotherapy has a role in the treatment of stones	5 <sup>th</sup> Experts in Stone Disease Conference	2024	Yes	Yes	No

July 28 – Aug 1 2024 Chicago USA	Using biomarkers in recurrent renal stone formers to detect silent stones	Association of Diagnostics & Laboratory Medicine 2024	2024	Yes	Yes*	No
Oct 23-26 2024 New Delhi, India	Temporal changes of selected urinary biomarkers in recurrent stone formers	44 <sup>th</sup> Congress of the Societe International d'Urologie	2024	Yes	Yes*	No

\*Acknowledgement of the research grant will be in the full presentation.

# 10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning

(Please elaborate)

Yes, undergraduate final year project students benefited to conduct tests and learn techniques

in ELISA, Urinalysis, and obtain data and do statistical analyses. Incorporated and updated

teaching of Analytical Chemistry, Toxicology and Research Methods in Medical Science.

# 11. Student(s) Trained

(Please attach a copy of the title page of the thesis)

N			Date of <del>Thesis</del>
Name	Degree Registered for	Date of Registration	Submission / Graduation
	BMSc Major in Forensic Science (Y1 Entry)	Sept. 2019	2023
	BMSc Major in Forensic Science (SY Entry)	Sept. 2021	2024
	BMSc Major in Forensic Science (SY Entry)	Sept. 2021	2024
	BMSc Major in Forensic Science (Y1 Entry)	Sept. 2000	2024
	BMSc Major in Forensic Science (Y1 Entry)	Sept. 2000	2024
	BMSc Major in Forensic Science (Y1 Entry)	Sept. 2019	2023

# 12. Other Impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

## Invited Plenary / Keynote Speaker

• "Debate – Phytotherapy has a role in the treatment of stones", Plenary lecture presented at the 5th Experts in Stone Disease Conference, Athens, Greece, June 10-11, 2022. (INVITED PLENARY LECTURE)

## Collaboration with other research institutions

- Prof. Anthony Ng Chi Fai, Dept. of Surgery, Prince of Wales Hospital, The Chinese University of Hong Kong, Hong Kong.
- Dr. John Yuen Wai Man, School of Nursing, The Hong Kong Polytechnic University, Hong Kong.
- Prof. Allen Rodgers, Dept. of Chemistry, University of Cape Town, Cape Town, South Africa.

# Editorial Board

• Appointed to the Editorial Board of "Urolithiasis", Springer-Verlag, Heidelberg, Germany.

# Teaching Enhancement

- 1 research staff recruited but stayed for 1-year due to CoVID limitations on working in hospital and the laboratory.
- Trained 6 undergraduate students to conduct ELISA, Urinalysis tests and data analyses alongside the PI.
- Incorporated the project proposal, statistics and literature review in the course "Research Methods in Medical Science" which is led by the PI.

## 13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	Conference Papers	Scholarly Books, Monographs and Chapters	Patents Awarded	Other Rese Output (please spe	arch s cify)
No. of outputs arising directly from this research project	2	4			Type Invited Plenary Lecture	No. 1

## 14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	Reasons		
N.A.			